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(54) Title: PATHOGEN TOLERANCE GENES

(57) Abstract: The present invention relates to transgenic plants and methods of making transgenic plant using punitive transcription factors that modulate the transgenic plant's susceptibility to disease.

PATHOGEN TOLERANCE GENES

RELATED APPLICATION INFORMATION

The present invention claims the benefit from US Provisional Patent Application Serial Nos. 60/166,228 filed November 17, 1999 and 60/197,899 filed April 17, 2000 and "Plant Trait Modification III" filed August 22, 2000.

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FIELD OF THE INVENTION

This invention relates to the field of plant biology. More particularly, the present invention pertains to compositions and methods for phenotypically modifying a plant.

BACKGROUND OF THE INVENTION

Transcription factors can modulate gene expression, either increasing or decreasing (inducing or repressing) the rate of transcription. This modulation results in differential levels of gene expression at various developmental stages, in different tissues and cell types, and in response to different exogenous (e.g., environmental) and endogenous stimuli throughout the life cycle of the organism.

Because transcription factors are key controlling elements of biological pathways, altering the expression levels of one or more transcription factors can change entire biological pathways in an organism. For example, manipulation of the levels of selected transcription factors may result in increased expression of economically useful proteins or metabolic chemicals in plants or to improve other agriculturally relevant characteristics. Conversely, blocked or reduced expression of a transcription factor may reduce biosynthesis of unwanted compounds or remove an undesirable trait. Therefore, manipulating transcription factor levels in a plant offers tremendous potential in agricultural biotechnology for modifying a plant's traits.

The present invention provides novel transcription factors useful for modifying a plant's phenotype in desirable ways, such as modifying a plant's pathogen tolerance.

SUMMARY OF THE INVENTION

In a first aspect, the invention relates to a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-29, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a); (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-29, or a complementary nucleotide sequence thereof; (d) a nucleotide sequence

comprising silent substitutions in a nucleotide sequence of (c); (e) a nucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a nucleotide sequence of one or more of: (a), (b), (c), or (d); (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e); (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's pathogen tolerance; (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g); (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g); (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29; (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29; and (1) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-29. The recombinant polynucleotide may further comprise a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence. The invention also relates to compositions comprising at least two of the above described polynucleotides.

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In a second aspect, the invention is an isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide described above.

In another aspect, the invention is a transgenic plant comprising one or more of the above described recombinant polynucleotides. In yet another aspect, the invention is a plant with altered expression levels of a polynucleotide described above or a plant with altered expression or activity levels of an above described polypeptide. Further, the invention may be a plant lacking a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-29.

The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

In a further aspect, the invention relates to a cloning or expression vector comprising the isolated or recombinant polynucleotide described above or cells comprising the cloning or expression vector.

In yet a further aspect, the invention relates to a composition produced by incubating a polynucleotide of the invention with a nuclease, a restriction enzyme, a polymerase; a polymerase and a primer; a cloning vector, or with a cell.

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Furthermore, the invention relates to a method for producing a plant having improved pathogen tolerance. The method comprises altering the expression of an isolated or recombinant polynucleotide of the invention or altering the expression or activity of a polypeptide of the invention in a plant to produce a modified plant, and selecting the modified plant for modified pathogen tolerance.

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In another aspect, the invention relates to a method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of the invention. The method comprises expressing a polypeptide encoded by the polynucleotide in a plant; and identifying at least one factor that is modulated by or interacts with the polypeptide. In one embodiment the method for identifying modulating or interacting factors is by detecting binding by the polypeptide to a promoter sequence, or by detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system, or by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

In yet another aspect, the invention is a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest. The method comprises placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of the invention and monitoring one or more of the expression level of the polynucleotide in the plant, the expression level of the polypeptide in the plant, and modulation of an activity of the polypeptide in the plant.

In yet another aspect, the invention relates to an integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of the invention, or to a polypeptide encoded by the polynucleotide. The integrated system, computer or computer readable medium may comprise a link between one or more sequence strings to a modified plant pathogen tolerance phenotype.

In yet another aspect, the invention is a method for identifying a sequence similar or homologous to one or more polynucleotides of the invention, or one or more polypeptides encoded by the polynucleotides. The method comprises providing a sequence database; and, querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

The method may further comprise of linking the one or more of the polynucleotides of the invention, or encoded polypeptides, to a modified plant pathogen tolerance phenotype.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

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Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

DETAILED DESCRIPTION

The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's pathogen tolerance when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered. Specifically, the polynucleotides and polypeptides are useful for modifying traits associated with a plant's pathogen tolerance, such as alterations in cell wall composition, trichome number or structure, callose induction, phytoalexin induction, alterations in the cell death response, or the like. Transgenic plants employing the polynucleotides or polypeptides of the invention are more tolerant to biotrophic or necrotrophic pathogens such as fungi, bacteria, mollicutes, viruses, nematodes, parasitic higher plants or the like.

The polynucleotides of the invention encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one or more of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) <u>J. Biol. Chem.</u> 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) <u>Trends Genet.</u> 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) <u>J. Biol.</u>

Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4:1575-1588); the miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes. Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 1996 250:7-16); the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-1 protein (Box P-binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides of the invention described herein, the polynucleotides and polypeptides of the invention have a variety of additional uses. These uses include their use in the recombinant production (i.e, expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, of as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

DEFINITIONS

A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA, a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either sense or antisense orientations.

A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

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An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polypucleotide. An "isolated polypeptide," whether a naturally occurring or a recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or additionally, the isolated polypeptide is separated from other cellular components with which it is typically associated, e.g., by any of the various protein purification methods herein.

The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to *in vitro* systems that mimic biochemical or cellular components or processes in a cell.

The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant

tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic is visible to the human eye, such as seed or plant size, or can be measured by available biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural observations such as stress tolerance, yield or pathogen tolerance.

"Trait modification" refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2%

increase or decrease in an observed trait (difference), at least a 5% difference, at least about a 10% difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution observed in wild type plant.

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Trait modifications of particular interest include those to seed (such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved tolerance to pest infestations, including nematodes, mollicutes, parasitic higher plants or the like; decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenyllipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that can be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time, flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

POLYPEPTIDES AND POLYNUCLEOTIDES OF THE INVENTION

The present invention provides, among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify a plant's pathogen tolerance.

Exemplary polynucleotides encoding the polypeptides of the invention were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence

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analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

Additional polynucleotides of the invention were identified by screening Arabidopsis thaliana and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate 5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in the Sequence Listing.

The polynucleotides of the invention were ectopically expressed in overexpressor or knockout plants and changes in the pathogen tolerance of the plants was observed. Therefore, the polynucleotides and polypeptides can be employed to improve the pathogen resistance of plants.

Making polynucleotides

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The polynucleotides of the invention include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., non-coding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or inteins, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

A variety of methods exist for producing the polynucleotides of the invention. Procedures for identifying and isolating DNA clones are well known to those of skill in the art, and are described in, e.g., Berger and Kimmel, <u>Guide to Molecular Cloning Techniques</u>, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA ("Berger");

Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

Alternatively, polynucleotides of the invention, can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis). Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, e.g., Ausubel, Sambrook and Berger, all supra.

Alternatively, polynucleotides and oligonucleotides of the invention can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically ligated to produce a desired sequence, e.g., a polynucleotide encoding all or part of a transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) Tetrahedron Letters 22:1859-69; and Matthes et al. (1984) EMBO J. 3:801-5. According to such methods, oligonucleotides are synthesized, purified, annealed to their complementary strand, ligated and then optionally cloned into suitable vectors. And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

HOMOLOGOUS SEQUENCES

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Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from Arabidopsis thaliana or from other plants of choice are also an aspect of the invention. Homologous sequences can be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn,

potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such pine, poplar and eucalyptus.

Transcription factors that are homologous to the listed sequences will typically share at least about 31% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level, the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the listed sequences. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

Identifying Nucleic Acids by Hybridization

Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50° C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription

factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.

SEQUENCE VARIATIONS

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It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides of the invention. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

Table 1

Amino acids			Codon			•		
Alanine	Ala		GCA	GCC	GCG	GCU		
Cysteine	Cys	C	TGC	TGT				
Aspartic acid	Asp	D	GAC	GAT				
Glutamic acid	Glu	E	GAA	GAG			:	
Phenylalanine	Phe	F	TTC	TTT		:		
Glycine	Gly	G	GGA	GGC	GGG	GGT		
Histidine	His	H	CAC	CAT	•			
Isoleucine	Ile	I	ATA	ATC	ATT			
Lysine	Lys	K	AAA -	AAG				
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT
Methionine	Met	M	ATG					
Asparagine	Asn	N	AAC	AAT				
Proline	Pro	P	CCA	CCC	CCG	CCT		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT
Serine	Ser	S	AGC	AGT	TCA	TCC	TCG	TCT
Threonine	Thr	T.	ACA	ACC	ACG.	ACT		
Valine	Val	V	GTA	GTC	GTG	GTT		
Tryptophan	Trp	W	TGG					
Tyrosine	Tyr	Y	TAC	TAT				

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Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art. Accordingly, any and all such variations of a sequence selected from the above table are a feature of the invention.

In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide, these conservative variants are, likewise, a feature of the invention.

For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) Meth. Enzymol. (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

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Table 2

Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Gln	Asn
Cys	Ser
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr; Gly
Thr	Ser;Val
Trp	Тут
Тут	Trp; Phe
Val	Ile; Leu

Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

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FURTHER MODIFYING SEQUENCES OF THE INVENTION—MUTATION/ FORCED EVOLUTION

In addition to generating silent or conservative substitutions as noted, above, the present invention optionally includes methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

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Thus, in one embodiment, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) Nature 370:389-391, and Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751. Many other mutation and evolution methods are also available and expected to be within the skill of the practitioner.

Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

Accordingly, the invention provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for *S. cerevisiae* and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and *E. coli* prefer to use TAA as the stop codon.

The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of

the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51; 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

EXPRESSION AND MODIFICATION OF POLYPEPTIDES

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Typically, polynucleotide sequences of the invention are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

Vectors, Promoters and Expression Systems

The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant

Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of Agrobacterium tumefaciens, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642, for dicotyledonous plants.

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Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou (1991) <u>Bio/Technology</u> 9: 957-962) and corn (Gordon-Kamm (1990) <u>Plant Cell</u> 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) <u>Plant Physiol</u> 102: 1077-1084; Vasil (1993) <u>Bio/Technology</u> 10: 667-674; Wan and Lemeaux (1994) <u>Plant Physiol</u> 104: 37-48, and for Agrobacterium-mediated DNA transfer (Ishida et al. (1996) <u>Nature Biotech</u> 14: 745-750).

Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

Examples of constitutive plant promoters which can be useful for expressing the TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (see, e.g., Odel et al. (1985) Nature 313:810); the nopaline synthase promoter (An et al. (1988) Plant Physiol 88:547); and the octopine synthase promoter (Fromm et al. (1989) Plant Cell 1: 977).

A variety of plant gene promoters that regulate gene expression in response to environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known promoters have been characterized and can favorable be employed to promote expression of a polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the dru 1 promoter (US Pat. No. 5,783,393), or the

2A11 promoter (US Pat. No. 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol Biol 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol Biol 37:977-988), flower-specific (Kaiser et al, (1995) Plant Mol Biol 28:231-243), pollen (Baerson et al. (1994) Plant Mol Biol 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson et al. (1993) Plant Mol Biol 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al. (1999) Plant Mol Biol 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinininducible promoter (Guevara-Garcia (1998) Plant Mol Biol 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol Biol 38:1053-1060, Willmott et al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley et al. (1993) Plant Mol Biol 22: 13-23), light (e.g., the pea rbcS-3A promoter, Kuhlemeier et al. (1989) Plant Cell 1:471, and the maize rbcS promoter, Schaffner and Sheen (1991) Plant Cell 3: 997); wounding (e.g., wunI, Siebertz et al. (1989) Plant Cell 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) Plant Mol. Biol. 40:387-396, and the PDF1.2 promoter described in Manners et al. (1998) Plant Mol. Biol. 38:1071-80), and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) Plant Mol Biol 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) Science 270: 1986-1988); or late seed development (Odell et al. (1994) Plant Physiol 106:447-458).

Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

Additional Expression Elements

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Specific initiation signals can aid in efficient translation of coding sequences. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation

codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

Expression Hosts

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The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention (including fragments thereof) by recombinant techniques. Host cells are genetically engineered (i.e, nucleic acids are introduced, e.g., transduced, transformed or transfected) with the vectors of this invention, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

The host cell can be a eukaryotic cell, such as a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Plant protoplasts are also suitable for some applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of Agrobacterium tumefaciens or A. rhizogenes carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by Agrobacterium tumefaciens, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

The cell can include a nucleic acid of the invention which encodes a polypeptide, wherein the cells expresses a polypeptide of the invention. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

For long-term, high-yield production of recombinant proteins, stable expression can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the

expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding mature proteins of the invention can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

Modified Amino Acids

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Polypeptides of the invention may contain one or more modified amino acids. The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production or modified by synthetic or chemical means.

Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids, biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

IDENTIFICATION OF ADDITIONAL FACTORS

A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a phentoype or trait of interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue of the invention is expressed in a host cell, e.g, a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After

identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al. (1999) Nature Biotechnology 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast Saccharomyces cerevisiae that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

IDENTIFICATION OF MODULATORS

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In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the

expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northerns, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) <u>Current Protocols in Molecular Biology</u>, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity. Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) Nature Biotechnology, 14(3):309-314 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. Science (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum C&EN Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, <u>Int. J. Pept. Prot. Res.</u> 37:487-493 (1991) and Houghton et al. <u>Nature 354:84-88 (1991)</u>). Other chemistries for generating chemical diversity libraries can also be used.

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In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

Indeed, entire high throughput screening systems are commercially available. These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators that have an effect on one or more polynucleotides or polypeptides according to the present invention.

In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with cells/plants/ etc. in one sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

In an embodiment, the invention provides a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell, plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In some cases, an alteration in a plant phenotype can be detected following contact of a plant (or plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

SUBSEQUENCES

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Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like, according to methods as noted *supra*.

Subsequences of the polynucleotides of the invention, including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides, or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

In addition, the invention includes an isolated or recombinant polypeptide including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides of the invention. For example, such polypeptides, or domains or fragments thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A

subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

PRODUCTION OF TRANSGENIC PLANTS

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Modification of Traits

The polynucleotides of the invention are favorably employed to produce transgenic plants with various traits, or characteristics, that have been modified in a desirable manner, e.g., to improve the pathogen resistance of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors (or transcription factor homologues) of the invention, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, improved pathogen tolerance, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

Antisense and Cosuppression Approaches

In addition to expression of the nucleic acids of the invention as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997) Antisense Technology: A Practical Approach IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various

least 30 nucleotides in length, and improved antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

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Vectors in which RNA encoded by a transcription factor or transcription factor homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to suppress expression of an endogenous transcription factor, thereby reducing or eliminating it's activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) Genes and Development 13: 139-141).

Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of Agrobacterium tumefaciens. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single

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transgene insertion event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) Methods in Arabidopsis Research, World Scientific).

Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) Nature 389:802).

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A plant trait can also be modified by using the cre-lox system (for example, as described in US Paent No. 5,658,772). A plant genome can be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides of the invention and/or expressing the polypeptides of the invention can be produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, of the invention, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledenous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean, clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcurbitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco,

peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) Handbook of Plant Cell Culture —Crop Species. Macmillan Publ. Co. Shimamoto et al. (1989) Nature 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

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Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; microinjection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and Agrobacterium tumeficiens mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

INTEGRATED SYSTEMS—SEQUENCE IDENTITY

Additionally, the present invention may be an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify sequences that meet any specified criteria. Furthermore, the instruction set may

be used to associate or link certain functional benefits, such improved pathogen tolerance, with one or more identified sequence.

For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madision, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched.

Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A description of the method is provided in Ausubel et al., supra.

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A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing sequence alignment are available, or can be produced by one of skill.

One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. J. Mol. Biol 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters

M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

The integrated system, or computer typically includes a user input interface allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

The methods of this invention can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may implemented on a single computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or

wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

Thus, the invention provides methods for identifying a sequence similar or homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a sequence database is provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

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Any sequence herein can be entered into the database, before or after querying the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

EXAMPLES

The following examples are intended to illustrate but not limit the present invention.

20 EXAMPLE I. FULL LENGTH GENE IDENTIFICATION AND CLONING

Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4 or -5 or lower, depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

Alternatively, Arabidopsis *thaliana* cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60° C) and labeled with ³²P dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO₄ pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60 °C with shaking. Filters were washed two times for 45 to 60 minutes with 1xSCC, 1% SDS at 60° C.

To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the MarathonTM cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the MarathonTM Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

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The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector 15 was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with SalI and NotI restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized 20 by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into competent cells of the E. coli strain 25 DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini Prep kits (Qiagen, CA).

EXAMPLE III. TRANSFORMATION OF AGROBACTERIUM WITH THE EXPRESSION VECTOR

After the plasmid vector containing the gene was constructed, the vector was used to transform Agrobacterium tumefaciens cells expressing the gene products. The stock of Agrobacterium tumefaciens cells for transformation were made as described by Nagel et al. (1990) FEMS Microbiol Letts. 67: 325-328. Agrobacterium strain ABI was grown in 250 ml

LB medium (Sigma) overnight at 28°C with shaking until an absorbance (A_{600}) of 0.5 – 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 µl chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125 µl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 µl and 750 µl, respectively. Resuspended cells were then distributed into 40 µl aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

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Agrobacterium cells were transformed with plasmids prepared as described above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50-100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 μ l of Agrobacterium cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 μ F and 200 μ F using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2-4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100μ g/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

EXAMPLE IV. TRANSFORMATION OF ARABIDOPSIS PLANTS WITH AGROBACTERIUM TUMEFACIENS WITH EXPRESSION VECTOR

After transformation of Agrobacterium tumefaciens with plasmid vectors containing the gene, single Agrobacterium colonies were identified, propagated, and used to transform Arabidopsis plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance (A_{600}) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044 μ M benzylamino purine (Sigma), 200 μ l/L Silwet L-77 (Lehle Seeds) until an absorbance (A_{600}) of 0.8 was reached.

Prior to transformation, Arabidopsis thaliana seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75 µE/m²/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of

multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

The pots were then immersed upside down in the mixture of Agrobacterium infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

EXAMPLE V. IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H₂O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H2O. The seeds were stored in the last wash water at 4°C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75 µE/m²/sec) at 22-23° C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T1 generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium).

Primary transformants were crossed and progeny seeds (T₂) collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level expression.

EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH TRANSCRIPTION FACTOR GENE KNOCKOUTS

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The screening of insertion mutagenized *Arabidopsis* collections for null mutants in a known target gene was essentially as described in Krysan et al (1999) <u>Plant Cell</u> 11:2283-2290. Briefly, gene-specific primers, nested by 5-250 bases to each other, were designed from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All possible combinations of gene specific and T-DNA/transposon primers were used to detect by PCR an insertion event within or close to the target gene. The amplified DNA fragments were then sequenced which allows the precise determination of the T-DNA/transposon insertion point relative to the target gene. Insertion events within the coding or intervening sequence of the genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique mutant plant for functional characterization. The method is described in more detail in Yu and Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

15 EXAMPLE VII. IDENTIFICATION OF PATHOGEN INDUCED GENES

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In some instances, expression patterns of the pathogen induced genes (such as defense genes) was monitored by microarray experiments. cDNAs were generated by PCR and resuspended at a final concentration of ~ 100 ng/ul in 3X SSC or 150mM Na-phosphate (Eisen and Brown (1999) *Meth. in Enzymol.* 303:179-205). The cDNAs were spotted on microscope glass slides coated with polylysine. The prepared cDNAs were aliquoted into 384 well plates and spotted on the slides using an x-y-z gantry (OmniGrid) purchased from GeneMachines (Menlo Park, CA) outfitted with quill type pins purchased from Telechem International (Sunnyvale, CA). After spotting, the arrays were cured for a minimum of one week at room temperature, rehydrated and blocked following the protocol recommended by Eisen and Brown (1999).

Sample total RNA (10 ug) samples were labeled using fluorescent Cy3 and Cy5 dyes. Labeled samples were resuspended in 4X SSC/0.03% SDS/4 ug salmon sperm DNA/2 ug tRNA/ 50mM Na-pyrophosphate, heated for 95°C for 2.5 minutes, spun down and placed on the array. The array was then covered with a glass coverslip and placed in a sealed chamber. The chamber was then kept in a water bath at 62°C overnight. The arrays were washed as described in Eisen and Brown (1999) and scanned on a General Scanning 3000 laser scanner. The resulting files are subsequently quantified using Imagene a software purchased from BioDiscovery (Los Angeles, CA).

EXAMPLE VIII. IDENTIFICATION OF PATHOGEN TOLERANCE PHENOTYPE IN OVEREXPRESSOR OR GENE KNOCKOUT PLANTS

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Experiments were performed to identify those transformants or knockouts that exhibited an improved pathogen tolerance. For such studies, the transformants were exposed to biotropic fungal pathogens, such as Erisyphe orontii, and necrotropic fungal pathogens, such as Fusarium oxysporum. Fusarium oxysporum isolates cause vascular wilts and damping off of various annual vegetables, perennials and weeds (Mauch-Mani and Slusarenko (1994) Molecular Plant-Microbe Interactions 7: 378-383). For Fusarium oxysporum experiments, plants grown on petri dishes were sprayed with a fresh spore suspension of F. oxysporum. The spore suspension was prepared as follows: A plug of fungal hyphae from a plate culture was placed on a fresh potato dextrose agar plate and allowed to spread for one week. 5 ml sterile water was then added to the plate, swirled, and pipetted into 50 ml Armstrong Fusarium medium. Spores were grown overnight in Fusarium medium and then sprayed onto plants using a Preval paint sprayer. Plant tissue was harvested and frozen in liquid nitrogen 48 hours post infection.

Erysiphe orontii is a causal agent of powdery mildew. For Erysiphe orontii experiments, plants were grown approximately 4 weeks in a greenhouse under 12 hour light (20 C, ~30% relative humidity (rh)). Individual leaves were infected with E. orontii spores from infected plants using a camel's hair brush, and the plants were transferred to a Percival growth chamber (20 C, 80% rh.). Plant tissue was harvested and frozen in liquid nitrogen 7 days post infection.

Botrytis cinerea is a necrotrophic pathogen. Botrytis cinerea was grown on potato dextrose agar in the light. A spore culture was made by spreading 10 ml of sterile water on the fungus plate, swirling and transferring spores to 10 ml of sterile water. The spore inoculum (approx. 105 spores/ml) was used to spray 10 day-old seedlings grown under sterile conditions on MS (-sucrose) media. Symptoms were evaluated every day up to approximately 1 week.

Infection with bacterial pathogens Pseudomonas syringae pv maculicola strain 4326 and pv maculicola strain 4326 was performed by hand inoculation at two doses. Two inoculation doses allows the differentiation between plants with enhanced susceptibility and plants with enhanced resistance to the pathogen. Plants were grown for 3 weeks in the greenhouse, then transferred to the growth chamber for the remainder of their growth. Psm ES4326 was hand inoculated with 1 ml syringe on 3 fully-expanded leaves per plant (4 1/2 wk old), using at least 9 plants per overexpressing line at two inoculation doses, OD=0.005 and OD=0.0005. Disease scoring occured at day 3 post-inoculation with pictures of the plants and leaves taken in parallel.

Table 3 shows the phenotypes observed for particular overexpressor or knockout plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or overexpressor plant was analyzed and the observed phenotype.

Table 3

SEQ ID No.	GID	Knockout (KO) or overexpressor (OE)	Phenotype
1	G188	KO	Increased susceptibility to Fusarium
3	G616	OE	Increased tolerance to Erysiphe
5	Gi9	OE	Increased tolerance to Erysiphe
7.	G261	OE	Increased susceptibility to Botrytis
9	G28	OE	Increased resistance to Erysiphe
11	G869	OE	Increased susceptibility to Fusarium
13	G237	OE	Increased tolerance to Erysiphe
15	G409	OE	Increased tolerance to Erysiphe
17	G418	OE	Increased tolerance to Pseudomonas
19	G591	OE	Increased tolerance to Erysiphe
21	G525	OE	Increased tolerance to Pseudomonas
23	G545	OE .	Increased susceptibility to Pseudomonas, Erysiphe and Fusarium
25	G865	OE	Increased susceptibility to Erysiphe and Botrytis
27	G881	OE	Increased susceptibility to Erysiphe and Botrytis
29	G896	KO	Increased susceptibility to Fusarium
31	G378	OE	Increased resistance to Erysiphe
33	G569	OE	Decreased expression of defense genes
35	G558	OE	Increased expression of defense genes

For a particular overexpressor that shows an increased susceptibility to a pathogen, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows an increased susceptibility to a pathogen, it may be more useful to select a plant with an increased expression of the particular transcription factor.

Other than Fusarium oxysporum, Erysyphe orontii, the transgenic plants are more tolerant to Sclerotinia spp., soil-borne oomycetes, foliar oomycetes, Botrytis spp., Rhizoctonia spp, Verticillium dahliae/albo-atrum, Alternaria spp., rusts, Mycosphaerella spp, Fusarium solani, or the like. The transgenic plants are more resistant to fungal diseases such as rusts, smuts, wilts, yellows, root rot, leaf drop, ergot, leaf blight of potato, brown spot of rice, leaf blight, late blight, powdery mildew, downy mildew, and the like; viral diseases such as sugarcane mosaic, cassava mosaic, sugar beet yellows, plum pox, barley yellow dwarf, tomato yellow leaf curl, tomato spotted wilt virus, and the like; bacterial diseases such as citrus canker, bacterial leaf blight, bacterial will, soft rot of vegetables, and the like; nematode diseases such as root knot, sugar beet cyst nematode or the like.

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EXAMPLE IX. IDENTIFICATION OF HOMOLOGOUS SEQUENCES

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Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) <u>J. Mol. Biol.</u> 215:403-410; and Altschul et al. (1997) <u>Nucl. Acid Res.</u> 25: 3389-3402). The tblastx sequence analysis programs were employed using the BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) <u>Proc. Natl. Acad. Sci. USA</u> 89: 10915-10919).

Identified *Arabidopsis* homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-58 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-58, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of 3.6e-40 is 3.6 x 10⁻⁴⁰. For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-Arabidopsis genes shown in Figure 3 and the Arabidopsis genes in the sequence listing are: SEQ ID No. 1: 38%-76%; SEQ ID No. 3: 36%-72%; SEQ ID No. 5: 51%-75%; SEQ ID No. 7: 37%-76%; SEQ ID No. 9: 48%-75%; SEQ ID No. 11: 31%-68%; SEQ ID No. 13: 59%-81%; SEQ ID No. 15: 49%-81%; SEQ ID No. 17: 53%-87%; SEQ ID No. 19: 48%-84%; SEQ ID No. 21: 73%-89%; SEQ ID No. 23: 52%-64%; SEQ ID No. 25: 48%-83%; SEQ ID No. 27: 35%-92%; SEQ ID No. 29: 56%-89%; SEQ ID No. 31: 50%-90%; SEQ ID No. 33: 50%-93%; SEQ ID No. 35: 52%-81%; SEQ ID No. 37: 75%-81%; SEQ ID No. 39: 35%-72%; SEQ ID No. 41: 55%-89%; SEQ ID No. 43: 56%-77%; SEQ ID No. 45: 34%-72%; SEQ ID No. 47: 51%-86%; SEQ ID No. 49: 46%-86%; SEQ ID No. 51: 58%-80%; SEQ ID No. 53: 46%-55%; SEQ ID No. 55: 84%-89%; and SEQ ID No. 57: 43%-71%.

The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the pathogen tolerance of a plant.

All references, publications, patents and other documents herein are incorporated by reference in their entirety for all purposes. Although the invention has been described with reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

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1. A transgenic plant with modified pathogen tolerance, which plant comprises a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- 5 (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-29, or a complementary nucleotide sequence thereof;
 - (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
- 10 (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-29, or a complementary nucleotide sequence thereof;
 - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
 - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
 - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
 - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's pathogen tolerance;
 - (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g);
 - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
- 25 (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29;
 - (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29; and
 - (1) a nucleotide sequence which encodes a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-29.
 - 2. The transgenic plant of claim 1, further comprising a constitutive, inducible, or tissue-active promoter operably linked to said nucleotide sequence.
- 35 3. The transgenic plant of claim 1, wherein the plant is selected from the group consisting of: soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot,

cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, and vegetable brassicas.

- 5 4. An isolated or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:
 - (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-29, or a complementary nucleotide sequence thereof;
- 10 (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);

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- (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-29, or a complementary nucleotide sequence thereof;
- (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
- (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
- (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
- 20 (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's pathogen tolerance;
 - (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g);
- 25 (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
 - (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEO ID Nos. 2N, where N=1-29;
 - (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29; and
 - (1) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-29.
- 35 5. The isolated or recombinant polynucleotide of claim 4, further comprising a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence.

6. A cloning or expression vector comprising the isolated or recombinant polynucleotide of claim 4.

- 7. A cell comprising the cloning or expression vector of claim 6.
- 8. A transgenic plant comprising the isolated or recombinant polynucleotide of claim 4.
- 9. A composition produced by one or more of:

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- (a) incubating one or more polynucleotide of claim 4 with a nuclease;
- 10 (b) incubating one or more polynucleotide of claim 4 with a restriction enzyme;
 - (c) incubating one or more polynucleotide of claim 4 with a polymerase;
 - (d) incubating one or more polynucleotide of claim 4 with a polymerase and a primer;
 - (e) incubating one or more polynucleotide of claim 4 with a cloning vector, or
 - (f) incubating one or more polynucleotide of claim 4 with a cell.
 - 10. A composition comprising two or more different polynucleotides of claim 4.
 - 11. An isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide of claim 4.
 - 12. A plant comprising an isolated polypeptide of claim 11.
 - 13. A method for producing a plant having a modified pathogen tolerance, the method comprising altering the expression of the isolated or recombinant polynucleotide of claim 4 or the expression levels or activity of a polypeptide of claim 11 in a plant, thereby producing a modified plant, and selecting the modified plant for improved pathogen tolerance thereby providing the modified plant with a modified pathogen tolerance.
 - 14. The method of claim 13, wherein the polynucleotide is a polynucleotide of claim 4.
 - 15. A method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of claim 4, the method comprising:
 - (a) expressing a polypeptide encoded by the polynucleotide in a plant; and
- (b) identifying at least one factor that is modulated by or interacts with the polypeptide.

16. The method of claim 15, wherein the identifying is performed by detecting binding by the polypeptide to a promoter sequence, or detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system.

- 5 17. The method of claim 15, wherein the identifying is performed by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.
 - 18. A method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest, the method comprising:
 - (a) placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of claim 4; and,
 - (b) monitoring one or more of:
 - (i) expression level of the polynucleotide in the plant;
 - (ii) expression level of the polypeptide in the plant;
 - (iii) modulation of an activity of the polypeptide in the plant; or
 - (iv) modulation of an activity of the polynucleotide in the plant.
 - 19. An integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of claim 4, or to a polypeptide encoded by the polynucleotide.
 - 20. The integrated system, computer or computer readable medium of claim 19, further comprising a link between said one or more sequence strings to a modified plant pathogen tolerance phenotype.

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- 21. A method of identifying a sequence similar or homologous to one or more polynucleotides of claim 4, or one or more polypeptides encoded by the polynucleotides, the method comprising:
 - (a) providing a sequence database; and,
- (b) querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.
- 35 22. The method of claim 21, wherein the querying comprises aligning one or more of the target sequences with one or more of the one or more sequence members in the sequence database.

23. The method of claim 21, wherein the querying comprises identifying one or more of the one or more sequence members of the database that meet a user-selected identity criteria with one or more of the target sequences.

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- 24. The method of claim 21, further comprising linking the one or more of the polynucleotides of claim 4, or encoded polypeptides, to a modified plant pathogen tolerance phenotype.
- 10 25. A plant comprising altered expression levels of an isolated or recombinant polynucleotide of claim 4.
 - 26. A plant comprising altered expression levels or the activity of an isolated or recombinant polypeptide of claim 11.

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27. A plant lacking a nucleotide sequence encoding a polypeptide of claim 11.

Figure 1

SEQ ID No.	GID	cDNA or protein	conserved domain
1	G188	cDNA	
2	G188	protein	175-222
3	G616	cDNA	
4	G616	protein	39-95
5	G19	cDNA	
6	G19	protein	76-145
7	G261	cDNA	
8	G261	protein	16-104
9	G28	cDŇA	
10	G28	protein	145-213
11	G869	cDNA	
12	G869	protein	109-177
13	G237	cDNA	
14	G237	protein	11-113
15	G409	cDNA	
16	G409	protein	64-124
17	G418	cDNA	
18	G418	protein	500-560
19	G591	cDNA _	
20	G591	protein	143-240
21	G525	cDNA	
22	G525	protein	23-167
23	G545	cDNA	
24	G545	protein	82-102, 136-154
25	G865	cDNA	·
26	G865	protein	36-103
27	G881	cDNA	
28	G881	protein	176-233
29	G896	cDNA	
30	G896	protein	18-39
31	G378	cDNA	,
32	G378	protein	196-237
33	G569	cDNA	
34	G569	protein	90-153
35	G558	cDNA	
36	G558	protein	45-105

Figure 2

SEQ ID No.	GID	homolog	cDNA or protein	conserved domain
37	G1396	homolog of G1394	cDNA	
38	G1396	homolog of G1394	protein	entire protein
39	G265	homolog of G261	cDNA	
40	G265	homolog of G261	protein	14-105
41	G1006	homolog of G28	cDNA	•
42	G1006	homolog of G28	protein	114-182
43	G1309	homolog of G237	cDNÀ	
44	G1309	homolog of G237	protein	9-114
45	G2550	homolog of G418	cDNA .	
46	G2550	homolog of G418	protein	348-408
47	G965	homolog of G418	cDNA	
48	G965	homolog of G418	protein	423-486
49	G793	homolog of G591	cDNA	
50	G793	homolog of G591	protein	151-206
51	G764	homolog of G525	cDNA	
52	G764	homolog of G525	protein	27-171
53	G350	homolog of G545	cDNA	
54	G350	homolog of G545	protein	91-113,150-170
55	G986	homolog of G881	cDNA	
56	G986	homolog of G881	protein	146-203
57	G1349	homolog of G896	cDNA	
58	G1349	homolog of G896	protein	13-63

Figure 3A

SEQ ID No.	GID	Genbank NID	P-value	Species
1	G188	7779802	5.20E-36	Lotus japonicus
1	G188	7284340	2.10E-34	Glycine max
1	G188	9361307	1.20E-27	Triticum aestivum
1	G188	7340336	1.10E-22	Oryza sativa
1	G188	6529152	3.60E-22	Lycopersicon esculentum
1	G188	8748477	7.70E-21	Medicago truncatula
1	G188	5456433	7.10E-14	Zea mays
1	G188	9302479	1.60E-12	Sorghum bicolor
1	G188	6696287	4.10E-12	Pinus taeda
1	G188	562242	9.00E-12	Brassica rapa
3	G616	7719440	8.30E-37	Lotus japonicus
3	G616	7692230	5.90E-33	Glycine max
3	G616	7501307	1.10E-21	Gossypium arboreum
3	G616	8071090	1.50E-21	Solanum tuberosum
3	G616	8858771	1.50E-21	Oryza sativa
3	G616	5047315	1.50E-21	Gossypium hirsutum
3	G616	6358532	5.80E-20	Antirrhinum graniticum
3	G616	2826867	7.00E-20	Antirrhinum majus
3	G616	6358535	7.40E-20	Antirrhinum majus subsp. linkianum
3	G616	6358538	7.50E-20	Antirrhinum braun-blanquetii
5	G19	8789223	2.80E-34	Citrus x paradisi
5	G19	9434234	4.50E-34	Lycopersicon esculentum
5	G19	7478682	1.30E-30	Glycine max
5	G19	6654934	1.20E-28	Medicago truncatula
5	G19	3264766	5.50E-26	Prunus armeniaca
. 5	G19	7624302	8.30E-26	Gossypium arboreum
5	G19	9425363	2.90E-25	Triticum aestivum
5	G19	688579	3.60E-25	Ricinus communis
5	G19	9419304	6.00E-25	Hordeum vulgare
5	G19	7720316	8.80E-25	Lotus japonicus
7	G261	5821137	5.10E-93	Nicotiana tabacum
7	G261	7158881	8.80E-86	Medicago sativa
7	G261	886741	1.00E-73	Zea mays
7	G261	5900449	5.20E-47	Lycopersicon esculentum
7	G261	7561318	1.20E-46	Medicago truncatula
7	G261	19491	1.70E-42	Lycopersicon peruvianum
7	G261	7233914	3.50E-41	Glycine max
7	G261	4528238	9.00E-41	Citrus unshiù
7	G261	8903922	4.00E-39	Hordeum vulgare
7	G261	9251913	1.90E-36	Solanum tuberosum
9	G28	7528275	4.20E-62	Mesembryanthemum crystallinum
9	G28	6654776	1.20E-57	Medicago truncatula
9	G28	790362	2.30E-54	Nicotiana tabacum
9	G28	8809570	8.00E-54	Nicotiana sylvestris
9	G28	3342210	8.40E-54	Lycopersicon esculentum
9	G28	6566281	8.40E-47	Glycine max
9	G28	7627061	8.40E-47	Gossypium arboreum
9	G28	7324479	2.00E-44	Lycopersicon pennellii
9	G28	6478844	1.80E-35	Matricaria chamomilla
9	G28	7273972	7.80E-29	Oryza sativa
11	G869	2213784	1.30E-19	Lycopersicon esculentum
11	G869	3065894	7.30E-19	Nicotiana tabacum

Figure 3B

SEQ ID No.	GID	Genbank NID	P-value	Species
11	G869	8570080	4.20E-18	Oryza sativa
11	G869	7560260	1.50E-17	Medicago truncatula
11	G869	7534890	5.20E-14	Sorghum bicolor
11	G869	6455322	1.10E-13	Glycine max
11	G869	9362061	2.70E-13	Triticum aestivum
11	G869	7788764	5.70E-13	Lotus japonicus
11	G869	7624302	2.50E-12	Gossypium arboreum
11	G869	3858036	2.80E-12	Populus balsamifera subsp. trichocarpa
13	G237	8283916	4.70E-42	Glycine max
13	G237	9361969	8.30E-41	Triticum aestivum
13	G237	4753385	4.10E-39	Zea mays
13	G237	7535969	4.10E-33	Sorghum bicolor
13	G237	7566043	9.30E-33	Medicago truncatula
13	G237	7339127	2.00E-32	Lycopersicon esculentum
13	G237	5860031	1.10E-28	Pinus taeda
13	G237	7776223	2.20E-28	Lotus japonicus
13	G237	6850206	5.10E-28	Oryza sativa
13	G237	5048991	8.50E-28	Gossypium hirsutum
15	G409	6654773	6.10E-57	Medicago truncatula
15	G409	6531235	2.00E-56	Lycopersicon esculentum
15	G409	7924152	1.10E-47	Glycine max
15	G409	5006854	6.50E-43.	Oryza sativa
15	G409	8098529	2.10E-41	Hordeum vulgare
15	G409	767697	1.40E-37	Daucus carota
15	G409	8328991	3.30E-37	Mesembryanthemum crystallinum
15	G409	7415613	1.40E-32	Physcomitrella patens
15	G409	7785121	2.80E-32	Lotus japonicus
15	G409	6916941	4.80E-32	Lycopersicon pennellii
17	G418	7239156	1.90E-123	Malus x domestica
17	G418	5892190	2.00E-62	Lycopersicon esculentum
17	G418	7628137	8.70E-58	Gossypium arboreum
17	G418	9205496	3.90E-51	Glycine max
17	G418	6069643	1.50E-45	Oryza sativa
17	G418	7562931	6.90E-45	Medicago truncatula
17	G418	7781695	5.50E-40	Lotus japonicus
17	G418	9298824	7.80E-34	Sorghum bicolor
17	G418	9428023	3.90E-32	Triticum aestivum
17	G418	7244366	1.30E-31	Mentha x piperita
19	G591	7646333	1.90E-55	Lycopersicon esculentum
19	G591	7924288	4.10E-53	Glycine max
19	G591	7722838	1.10E-41	Lotus japonicus
19	G591	5804781	1.40E-24	Nicotiana tabacum
19	G591	9198126	2.50E-23	Medicago truncatula
19	G591	427677	9.50E-15	Oryza sativa
19	G591	7624745	1.80E-14	Gossypium arboreum
19	G591	7535578	8.70E-14	Sorghum bicolor
19	G591	5915205	1.30E-11	Zea mays
19	G591	9249806	2.60E-11	Solanum tuberosum
21	G525	4384535	5.60E-61	Lycopersicon esculentum
21	G525	6454868	2.00E-58	Glycine max
21	G525	6066594	9.30E-54	Petunia x hybrida
21	G525	4977542	8.60E-51	Oryza sativa

Figure 3C

SEQ ID No.	GID	Genbank NID	P-value	Species
21	G525	9361647	2.50E-50	Triticum aestivum
21	G525	4218536	5.20E-50	Triticum sp.
21	G525	6732159	5.20E-50	Triticum monococcum
21	G525	5343151	2.70E-49	Zea mays
21	G525	5049217	4.20E-48	Gossypium hirsutum
21	G525	8708684	8.90E-48	Hordeum vulgare
23	G545	4666359	8.30E-55	Datisca glomerata
23	G545	7228328	3.70E-52	Medicago sativa
23	G545	1763062	1.30E-51	Glycine max
23	G545	7206360	3.10E-44	Medicago truncatula
23	G545	7626808	9.60E-40	Gossypium arboreum
23	G545	439492	3.90E-39	Petunia x hybrida
23	G545	4382658	1.70E-38	Lycopersicon esculentum
23	G545	8486215	8.70E-38	Euphorbia esula
23	G545	7322653	6.80E-37	Lycopersicon hirsutum
23	G545	7785845	1.10E-33	Lotus japonicus
25	G865	9417297	1.70E-32	Triticum aestivum
25	G865	7206394	4.90E-29	Medicago truncatula
25	G865	7796858	5.70E-27	Glycine max
25	G865	4387560	9.20E-25	Lycopersicon esculentum
25	G865	569065	1.50E-23	Oryza sativa
25	G865	7788764	4.10E-23	Lotus japonicus
25	G865	790362	8.40E-22	Nicotiana tabacum
25	G865	7528275	5.90E-21	Mesembryanthemum crystallinum
25	G865	3264766	8.80E-20	Prunus armeniaca
25	G865	8098026	2.00E-19	Hordeum vulgare
27	G881	5820418	9.80E-29	Glycine max
27	G881	8440065	1.00E-27	Gossypium hirsutum
27	G881	4380578	1.50E-27	Lycopersicon esculentum
27	G881	9199620	2.70E-27	Medicago truncatula
27	G881	6472584	2.20E-24	Nicotiána tabacum
27	G881	9250698	3.20E-24	Solanum tuberosum
27	G881	8205146	5.20E-21	Oryza sativa
27	G881	1159878	8.20E-17	Avena fatua
27	G881	9299778	2.70E-16	Sorghum bicolor
27	G881	9444636	1.10E-14	Triticum aestivum
29	G896	9410462	1.90E-101	Hordeum vulgare
29	G896	7628908	3.60E-82	Gossypium arboreum
29	G896	7244408	1.80E-79	Mentha x piperita
29	G896	5046180	2.10E-73	Gossypium hirsutum
29	G896	7678652	1.10E-63	Lotus japonicus
29	G896	8286031	1.40E-60	Glycine max
29	G896	5888938	4.50E-58	Lycopersicon esculentum
29	G896	9298238	9.20E-54	Sorghum bicolor
29	G896	7566414	8.00E-52	Medicago truncatula
29	G896	8845076	1.00E-46	Triticum aestivum
31	G378	5270028	5.10E-73	Lycopersicon esculentum
31	G378	5048335	4.10E-58	Gossypium hirsutum
31	G378	7239521	5.90E-42	Oryza sativa
31	G378	5606120	6.80E-36	Glycine max
31	G378	3853800	3.20E-30	Populus tremula x Populus tremuloides
31	G378	7659983	1.70E-23	Sorghum bicolor
<u> </u>	00/0	, , , , , , , , , , , , , , , , , , , ,	1 35-20	Tan Bushin propriet

Figure 3D

SEQ ID No.	GID	Genbank NID	P-value	Species
31	G378	6626305	1.10E-21	Zea mays
31	G378	9412941	9.40E-19	Triticum aestivum
31	G378	3242033	4.30E-17	Mesembryanthemum crystallinum
31	G378	7626259	7.70E-13	Gossypium arboreum
33	G229	7337390	6.60E-51	Lycopersicon esculentum
33	G229	9823237	3.60E-50	Hordeum vulgare
33	G229	7244424	4.90E-50	Mentha x piperita
33	G229	7776053	1.70E-49	Lotus japonicus
33	G229	2921335	5.80E-48	Gossypium hirsutum
33	G229	1491932	4.50E-47	Zea mays
33	G229	6455590	2.80E-44	Glycine max
33	G229	6020191	2.00E-41	Pinus taeda
33	G229	10697236	4.20E-41	Oryza sativa
33	G229	7765706	5.10E-41	Medicago truncatula
35	G663	7673087	5.10E-43	Petunia integrifolia
35	G663	9508051	3.00E-41	Lycopersicon esculentum
35	G663	7673091	3.30E-41	Petunia x hybrida
			2.40E-36	Petunia axillaris
35	G663	7673097		
35	G663	5048991	1.20E-33 2.50E-31	Gossypium hirsutum
35	G663	6455590		Glycine max
35	G663	7560175	1.90E-27	Medicago truncatula
35	G663	7244424	4.10E-26	Mentha x piperita
35	G663	9954117	3.40E-25	Solanum tuberosum
35	G663_	6020191	3.60E-25	Pinus taeda
37	G1396	498704	5.20E-22	Spinacia oleracea
37	G1396	7502400	1.20E-21	Gossypium arboreum
37	G1396	3857536	3.40E-21	Populus balsamifera subsp. trichocarpa
37	G1396	4385300	1.20E-20	Lycopersicon esculentum
37	G1396	6917249	1.50E-20	Lycopersicon pennellii
37	G1396	6915979	1.70E-20	Glycine max
37	G1396	7674530	2.70E-20	Medicago truncatula
37	G1396	8090319	3.40E-20	Sorghum bicolor
37	G1396	3592182	9.10E-20	Oryza sativa
37	G1396	6654124	1.10E-19	Zea mays
39	G265	5821137	6.50E-83	Nicotiana tabacum
39	G265	7158881	3.80E-79	Medicago sativa
39	G265	886741	1.60E-70	Zea mays
39	G265	5900449	5.60E-43	Lycopersicon esculentum
39	G265	8903922	8.20E-43	Hordeum vulgare
39	G265	7561318	2.10E-41	Medicago truncatula
39	G265	9204445	5.30E-36	Glycine max
. 39	G265	4528238	5.40E-36	Citrus unshiu
39	G265	19489	2.10E-35	Lycopersicon peruvianum
39	G265	9251913	2.00E-32	Solanum tuberosum
41	G1006	7528275	2.70E-51	Mesembryanthemum crystallinum
41	G1006	3342210	4.90E-49	Lycopersicon esculentum
41	G1006	6654776	1.90E-48	Medicago truncatula
41	G1006	790362	2.30E-47	Nicotiana tabacum
41	G1006	8809570	2.00E-46	Nicotiana sylvestris
41	G1006	7627061	6.40E-41	Gossypium arboreum
41	G1006	7324479	1.20E-35	Lycopersicon pennellii
41	G1006	6478844	1.80E-35	Matricaria chamomilla

Figure 3E

SEQ ID No.	GID	Genbank NID	P-value	Species
41	G1006	6566281	1.30E-34	Glycine max
41	G1006	4716624	3.80E-28	Oryza sativa
43	G1309	9361969	2.40E-45	Triticum aestivum
43	G1309	7566043	9.60E-35	Medicago truncatula
43	G1309	5891104	2.20E-31	Lycopersicon esculentum
43	G1309	5860031	2.10E-30	Pinus taeda
43	G1309	5049507	6.20E-30	Gossypium hirsutum
43	G1309	5139805	1.30E-29	Glycine max
43	G1309	6850206	2.50E-29	Oryza sativa
43	G1309	7721017	3.40E-29	Lotus japonicus
43	G1309	8368245	5.20E-28	Zea mays
43	G1309	20560	9.50E-27	Petunia x hybrida
45	G2550	4380729	2.80E-51	Lycopersicon esculentum
45	G2550	5667196	2.20E-49	Oryza sativa
45	G2550	8669454	1.40E-48	Glycine max
45	G2550	9298824	1.50E-48	Sorghum bicolor
45	G2550	7239156	9.90E-46	Malus x domestica
45	G2550	7570704	5.70E-45	Medicago truncatula
45	G2550	7628137	3.30E-42	Gossypium arboreum
45	G2550	7244366	6.00E-41	Mentha x piperita
45	G2550	9428023	4.70E-40	Triticum aestivum
45	G2550	9250642	3.50E-39	Solanum tuberosum
47	G965	7239156	3.10E-126	Malus x domestica
47	G965	5892190	2.00E-62	Lycopersicon esculentum
47	G965	7628137	1.60E-56	Gossypium arboreum
47	G965	9205496	2.60E-49	Glycine max
47	G965	6069643	1.70E-45	Oryza sativa
47	G965	7562931	2.50E-44	Medicago truncatula
47	G965	7781695	1.60E-41	Lotus japonicus
47	G965	9298824	6.30E-33	Sorghum bicolor
47	G965	9428023	1.50E-31	Triticum aestivum
47	G965	7244366	1.20E-29	Mentha x piperita
49	G793	6976712	3.60E-43	Lycopersicon esculentum
49	G793	7924288	2.00E-41	Glycine max
49	G793	7614163	3.90E-34	Lotus japonicus
49	G793	9198126	5.70E-23	Medicago truncatula
49	G793	5804781	1.10E-22	Nicotiana tabacum
49	G793	7535578	1.60E-14	Sorghum bicolor
49	G793	427677	6.10E-14	Oryza sativa
49	G793	5915205	2.90E-10	Zea mays
49	G793	9249806	4.20E-10	Solanum tuberosum
49	G793	7624745	1.30E-09	Gossypium arboreum
51	G764	4384535	7.00E-70	Lycopersicon esculentum
51	G764	5049217	1.80E-65	Gossypium hirsutum
51	G764	6454868	1.90E-64	Glycine max Petunia x hybrida
51	G764	6066594	5.20E-59	
51	G764	4218536	2.30E-52	Triticum sp. Triticum monococcum
51	G764	6732159	2.30E-52	Triticum monococcum Triticum aestivum
51	G764	9361647	7.50E-52	Oryza sativa
51	G764	4977542	4.10E-49	Medicago truncatula
51	G764	6799764	4.40E-49	Sorghum bicolor
51	G764	9296257	1.00E-48	Landin nicoloi

Figure 3F

SEQ ID No.	GID	Genbank NID	P-value	Species
53	G350	439492	5.20E-53	Petunia x hybrida
53	G350	7228328	8.90E-51	Medicago sativa
53	G350	4666359	3.10E-48	Datisca glomerata
53	G350	1763062	8.30E-48	Glycine max
53	G350	7626808	9.10E-44	Gossypium arboreum
53	G350	7206360	2.20E-43	Medicago truncatula
53	G350	2981168	2.10E-38	Nicotiana tabacum
53	G350	7322653	2.00E-37	Lycopersicon hirsutum
53	G350	5276755	2.40E-37	Lycopersicon esculentum
53	G350	2058503	1.10E-31	Brassica rapa
55	G986	6472584	1.00E-34	Nicotiana tabacum
55	G986	8440065	8.80E-33	Gossypium hirsutum
55	G986	4385167	1.50E-32	Lycopersicon esculentum
55	G986	8205146	5.50E-30	Oryza sativa
55	G986	5820418	8.80E-26	Glycine max
55	G986	1159878	2.30E-23	Avena fatua
55	G986	9250698	4.60E-22	Solanum tuberosum
55	G986	9413507	7.90E-21	Triticum aestivum
55	G986	7748539	2.30E-20	Lotus japonicus
55	G986	9199620	1.30E-16	Medicago truncatula
57	G1349	8904043	1.50E-47	Hordeum vulgare
57	G1349	7244408	2.40E-47	Mentha x piperita
57	G1349	8286031	3.60E-46	Glycine max
57	G1349	9298238	9.10E-36	Sorghum bicolor
57	G1349	7628908	4.70E-34	Gossypium arboreum
57	G1349	5046180	1.50E-33	Gossypium hirsutum
57	G1349	5888938	1.30E-30	Lycopersicon esculentum
57	G1349	5043924	6.20E-30	Pinus taeda
57	G1349	8845076	4.40E-29	Triticum aestivum
57	G1349	7678652	4.20E-27	Lotus japonicus

MBI15 Sequence Listing.ST25 SEQUENCE LISTING

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Pro Ile Lys Gly Ser Pro Tyr Pro Arg Asn Tyr Tyr Arg Cys Ser Ser 180 185 190

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MBI15 Sequence Listing.ST25

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MBI15 Sequence Listing.ST25 225 230 235

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MBI15 Sequence Listing.ST25

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Gln Leu Ala Lys Lys Leu Gly Leu Gln Pro Arg Gln Val Ala Val Trp

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MBI15 Sequence Listing.ST25

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-	==	Ξ.					4			· · ·	~ ~ ~	A =		cgt Arg	~1 -	1554
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cta Leu	t tg Leu	gct Ala 535	cga Arg	cag Gln	act Thr	ggt Gly	tta Leu 540	tcc Ser	aga Arg	aat Asn	cag Gln	gta Val 545	tca Ser	aat Asn	tgg Trp	1746
ttc Phe	ata Ile	aat Asn	gct Ala	agg Arg	gtt Val	cgt Arg	tta Leu	tgg Trp	Lys	cca Pro age	Met	gtg Val	gaa Glu	gaa Glu	atg Met	1794

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gac gct tca tca ctt ct Asp Ala Ser Ser Leu Le 645	u Leu Pro Ser Ser Tyr		2082
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gcg ttt tcc gcc gtt gc Ala Phe Ser Ala Val Al 680			2178
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Ser Asp Ser Pro Asn Leu Thr Thr Gln Gln Lys Gln Glu His Gln Arg Page 24 MBI15 Sequence Listing.ST25 50 55 60

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Tyr Glu Ser Ala Gly Met Leu Ser Glu Met Phe Asn Phe Pro Gly Ser 85 90 95

Ser Gly Gly Gry Arg Asp Leu Asp Leu Gly Gln Ser Phe Arg Ser Asn 100 105 110

Arg Gln Leu Leu Glu Glu Gln His Gln Asn Ile Pro Ala Met Asn Ala 115 120 125

Thr Asp Ser Ala Thr Ala Thr Ala Ala Ala Met Gln Leu Phe Leu Met 130 135 140

Asn Pro Pro Pro Pro Gln Gln Pro Pro Ser Pro Ser Ser Thr Thr Ser 145 150 155 160

Pro Arg Ser His His Asn Ser Ser Thr Leu His Met Leu Leu Pro Ser 165 170 175

Pro Ser Thr Asn Thr Thr His His Gln Asn Tyr Thr Asn His Met Ser

Met His Gln Leu Pro His Gln His His Gln Gln Ile Ser Thr Trp Gln 195 200 205

Ser Ser Pro Asp His His His His His His Asn Ser Gln Thr Glu Ile 210 215 220

Gly Thr Val His Val Glu Asn Ser Gly Gly His Gly Gly Gln Gly Leu 225 230 235 240

Ser Leu Ser Leu Ser Ser Ser Leu Glu Ala Ala Ala Lys Ala Glu Glu 245 250 255

Tyr Arg Asn Ile Tyr Tyr Gly Ala Asn Ser Ser Asn Ala Ser Pro His 260 265 270

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His His Gln Val Leu Asn Gln Phe Arg Ser Ser Pro Ala Ala Ser Ser 290 295 300

Ser Ser Met Ala Ala Val Asn Ile Leu Arg Asn Ser Arg Tyr Thr Thr 305 310 315 320

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Leu Lys Lys Asn Lys Leu Gly Asn Ser Ser Asn Pro Asn Thr Cys Gly 340 345 350

MBI15 Sequence Listing.ST25
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- Val Lys Leu Leu Thr Met Leu Glu Glu Val Asp Arg Arg Tyr Asn His 385 390 395 400
- Tyr Cys Glu Gln Met Gln Met Val Val Asn Ser Phe Asp Ile Val Met 405 410 415
- Gly His Gly Ala Ala Leu Pro Tyr Thr Ala Leu Ala Gln Lys Ala Met 420 425 430
- Ser Arg His Phe Arg Cys Leu Lys Asp Ala Val Ala Ala Gln Leu Lys 435 440 445
- Gln Ser Cys Glu Leu Leu Gly Asp Lys Asp Ala Ala Gly Ile Ser Ser 450 455 460
- Ser Gly Leu Thr Lys Gly Glu Thr Pro Arg Leu Arg Leu Leu Glu Gln 465 470 480
- Ser Leu Arg Gln Gln Arg Ala Phe His Gln Met Gly Met Met Glu Gln 485 490 495
- Glu Ala Trp Arg Pro Gln Arg Gly Leu Pro Glu Arg Ser Val Asn Ile 500 505 510
- Leu Arg Ala Trp Leu Phe Glu His Phe Leu His Pro Tyr Pro Ser Asp 515 520 525
- Ala Asp Lys His Leu Leu Ala Arg Gln Thr Gly Leu Ser Arg Asn Gln 530 540
- Val Ser Asn Trp Phe Ile Asn Ala Arg Val Arg Leu Trp Lys Pro Met 545 550 560
- Val Glu Glu Met Tyr Gln Gln Glu Ser Lys Glu Arg Glu Arg Glu Glu 565 570 575
- Glu Leu Glu Glu Asn Glu Glu Asp Gln Glu Thr Lys Asn Ser Asn Asp 580 585 590
- Asp Lys Ser Thr Lys Ser Asn Asn Asn Glu Ser Asn Phe Thr Ala Val 595 600 605
- Arg Thr Thr Ser Gln Thr Pro Thr Thr Thr Ala Pro Asp Ala Ser Asp 610 620
- Ala Asp Ala Ala Val Ala Thr Gly His Arg Leu Arg Ser Asn Ile Asn 625 630 630
- Ala Tyr Glu Asn Asp Ala Ser Ser Leu Leu Leu Pro Ser Ser Tyr Ser 645 650 655

MBI15 Sequence Listing.ST25

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Arg Phe Ser Asp Asp Val Val Asp Asn Arg Cys Ser Ser Met Lys Pro
110 115 120

gtt ttc cac ggg cag cct atg caa cag cca cct cca tcg gcc cca cat
Val Phe His Gly Gln Pro Met Gln Gln Pro Pro Pro Ser Ala Pro His
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Pro Pro Met Met Leu Gln Leu Gly Ser Gly Glu Glu Gly Ser His Met 50 60

MBI15 Sequence Listing.ST25

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Pro Glu Gly Gly His Gly Ser Gly Lys Arg Phe Ser Asp Asp Val Val

Asp Asn Arg Cys Ser Ser Met Lys Pro Val Phe His Gly Gln Pro Met

Gln Gln Pro Pro Pro Ser Ala Pro His Gln Pro Thr Ser Ile Arg Pro

Arg Val Arg Ala Arg Arg Gly Gln Ala Thr Asp Pro His Ser Ile Ala 145 150 150 155

Glu Arg Leu Arg Glu Arg Ile Ala Glu Arg Ile Arg Ala Leu Gln 165 170 175

Glu Leu Val Pro Thr Val Asn Lys Thr Asp Arg Ala Ala Met Ile Asp 180 185 190

Glu Ile Val Asp Tyr Val Lys Phe Leu Arg Leu Gln Val Lys Val Leu

Ser Met Asn Arg Leu Gly Gly Ala Gly Ala Val Ala Pro Leu Val Thr 210 215 220

Asp Met Pro Leu Ser Ser Ser Val Glu Asp Glu Thr Gly Glu Gly Gly 225 230 230 235

Arg Thr Pro Gln Pro Ala Trp Glu Lys Trp Ser Asn Asp Gly Thr Glu 245 250 255

Arg Gln Val Ala Lys Leu Met Glu Glu Asn Val Gly Ala Ala Met Gln 265 270

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			gaa gaa ctc ata act Glu Glu Leu Ile Thr 35	213
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			ttc tgt gtg aga gac Phe Cys Val Arg Asp 80	357
			gcg aca gaa gcc ggt Ala Thr Glu Ala Gly 95	405
			ttc aag gga aaa tca Phe Lys Gly Lys Ser 115	453
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			aag aac gaa tgg gtt Lys Asn Glu Trp Val . 160	597
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Ser Met Leu Asp	Pro His Ile Asn		cca gcc ggt tta cct Pro Ala Gly Leu Pro 195	693
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MBI15 Sequence Listing.ST25

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Ala Thr Ala Ile Gly Glu Val Asp Leu Asn Lys Ile Glu Pro Trp Asp 50 55 60

Leu Pro Trp Lys Ala Lys Met Gly Glu Lys Glu Trp Tyr Phe Phe Cys 65 75 80

Val Arg Asp Arg Lys Tyr Pro Thr Gly Leu Arg Thr Asn Arg Ala Thr 85 90 95

Glu Ala Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe Lys

Gly Lys Ser Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr Lys Gly

Arg Ala Pro Lys Gly Val Lys Thr Asn Trp Val Met His Glu Tyr Arg 130 135 140

Leu Glu Gly Lys Tyr Cys Ile Glu Asn Leu Pro Gln Thr Ala Lys Asn 145 150 160

Glu Trp Val Ile Cys Arg Val Phe Gln Lys Arg Ala Asp Gly Thr Lys 165 170 175

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MBI15 Sequence Listing.ST25

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Asp	Gly	Gln	Glu 260	Thr	Gln	Phe	Ser	Gly 265	Lys	Pro	Phe	Asp	Gly 270	Arg	Asp	
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					tca Ser											153
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					cgt Arg											393
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act Thr 130	gga Gly	agt Ser	999 Gly	aaa Lys	tca Ser 135	cac His	gtt Val	tgc Cys	acc Thr	atc Ile 140	tgt Cys	aac Asn	aag Lys	tct Ser	ttt Phe 145	489
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Asp 65	Asn	Arg	Gln	Pro	Pro 70	Pro	Pro	Pro	Ala	Val 75	Glu	Lys	Leu	Ser	Tyr 80	
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PCT/US00/31418

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180

728

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140

MBI15 Sequence Listing.ST25

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Gln Pro Arg Arg Arg His Tyr Arg Gly Val Arg Gln Arg Pro Trp Gly 35 40

Lys Trp Ala Ala Glu Ile Arg Asp Pro Lys Lys Ala Ala Arg Val Trp 50 55 60

Leu Gly Thr Phe Glu Thr Ala Glu Glu Ala Ala Leu Ala Tyr Asp Arg 65 70 80

Ala Ala Leu Lys Phe Lys Gly Thr Lys Ala Lys Leu Asn Phe Pro Glu 85 90 95

Arg Val Gln Gly Pro Thr Thr Thr Thr Thr Ile Ser His Ala Pro Arg

Gly Val Ser Glu Ser Met Asn Ser Pro Pro Pro Arg Pro Gly Pro Pro 115 120 125

Ser Thr Thr Thr Thr Ser Trp Pro Met Thr Tyr Asn Gln Asp Ile Leu 130 135 140

Gln Tyr Ala Gln Leu Leu Thr Ser Asn Asn Glu Val Asp Leu Ser Tyr

Tyr Thr Ser Thr Leu Phe Ser Gln Pro Phe Ser Thr Pro Ser Ser Ser Ser 165 170 175

MBI15 Sequence Listing.ST25

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1 10 111 ctc aac acc aat cct ttc tcc gca aaa ctt ccg aag aag gag gtc tca 159 Leu Asn Thr Asn Pro Phe Ser Ala Lys Leu Pro Lys Lys Glu Val Ser gtt ttg gct tct act cac tta aag agg aaa tgg ttg gag caa gac gag Val Leu Ala Ser Thr His Leu Lys Arg Lys Trp Leu Glu Gln Asp Glu 207 agc gca agt gag tta cga gag gag cta aac aga gtt aat tca gag aac Ser Ala Ser Glu Leu Arg Glu Glu Leu Asn Arg Val Asn Ser Glu Asn aag aag cta aca gag atg tta gct aga gtc tgt gag agc tac aac gaa Lys Lys Leu Thr Glu Met Leu Ala Arg Val Cys Glu Ser Tyr Asn Glu 65 70 75 303 cta cat aat cat ttg gag aag ctt cag agt cgc cag agc cct gaa atc Leu His Asn His Leu Glu Lys Leu Gln Ser Arg Gln Ser Pro Glu Ile 351 399 gag cag acc gat ata ccg ata aag aaa aga aaa caa gac ccg gat gag Glu Gln Thr Asp Ile Pro Ile Lys Lys Arg Lys Gln Asp Pro Asp Glu ttc tta ggc ttt cct att gga ctc agt agt gga aaa act gag aac agc Phe Leu Gly Phe Pro Ile Gly Leu Ser Ser Gly Lys Thr Glu Asn Ser 110 115 120 447 tcc agc aac gaa gat cat cat cat cat cat cag caa cat gag cag aaa Ser Ser Asn Glu Asp His His His His Gln Gln His Glu Gln Lys 495 130 aat cag ctt ctt tca tgt aaa aga cca gtc act gat agc ttc aac aaa Asn Gln Leu Leu Ser Cys Lys Arg Pro Val Thr Asp Ser Phe Asn Lys 543 gca aaa gtt tcg act gtc tac gtg cct act gaa aca tcg gac aca agc Ala Lys Val Ser Thr Val Tyr Val Pro Thr Glu Thr Ser Asp Thr Ser 591 165

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639

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180

MBI15 Sequence Listing.ST25

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Leu Arg Glu Glu Leu Asn Arg Val Asn Ser Glu Asn Lys Lys Leu Thr 50 55

Glu Met Leu Ala Arg Val Cys Glu Ser Tyr Asn Glu Leu His Asn His 65 70 75 80

Leu Glu Lys Leu Gln Ser Arg Gln Ser Pro Glu Ile Glu Gln Thr Asp 90 95

Ile Pro Ile Lys Lys Arg Lys Gln Asp Pro Asp Glu Phe Leu Gly Phe 100 105 110

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Ser 145	Сув	Lys	Arg	Pro	Val 150	Thr	Asp	Ser	Phe	Asn 155	Lys	Ala	Lys	Val	Ser 160	
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Pro	Ser	Pro 195	Arg	Ala	Tyr	Phe	Arg 200	Сув	Ser	Phe	Ala	Pro 205	Ser	Сув	Pro	
Val	Lys 210	Lys	Lys	Val	Gln	Arg 215	Ser	Ala	Glu	Asp	Pro 220	Ser	Leu	Leu	Val	
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ggt Gly 20	tgc Cys	cgg Arg	acg Thr	cct Pro	ctc Leu 25	cag Gln	ctc Leu	cca Pro	tcc Ser	ggc Gly 30	gcc Ala	cga Arg	tct Ser	att Ile	cgc Arg 35	151

MBI15 Sequence Listing.ST25

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gat Asp 180	gaa Glu	aca Thr	ctc Leu	tgt Cys	cct Pro 185	ctg Leu	gat Asp	ttt Phe	gaa Glu	act Thr 190	cag Gln	ggg Gly	atg Met	att Ile	gta Val 195	631
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340 350 355

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Arg Thr Ala Pro Pro Pro Gln Pro Ser Ser Ala Pro Ser Pro Pro Pro 50 55 60

Gln Ile His Ala Pro Pro Gly Gln Leu Pro His Pro His Gly Arg Lys 65 70 75 80

Arg Ala Val Ile Cys Gly Ile Ser Tyr Arg Phe Ser Arg His Glu Leu 85 90 95

Lys Gly Cys Ile Asn Asp Ala Lys Cys Met Arg His Leu Leu Ile Asn 100 105 110

Lys Phe Lys Phe Ser Pro Asp Ser Ile Leu Met Leu Thr Glu Glu Glu 115 120 125

Thr Asp Pro Tyr Arg Ile Pro Thr Lys Gln Asn Met Arg Met Ala Leu 130 135 140

Tyr Trp Leu Val Gln Gly Cys Thr Ala Gly Asp Ser Leu Val Phe His 145 150 155 160

Tyr Ser Gly His Gly Ser Arg Gln Arg Asn Tyr Asn Gly Asp Glu Val 165 170 175

Asp Gly Tyr Asp Glu Thr Leu Cys Pro Leu Asp Phe Glu Thr Gln Gly 180 185 190

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His Gly Val Lys Leu His Ser Ile Ile Asp Ala Cys His Ser Gly Thr Page 40

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MBI15 Sequence Listing.ST25

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Gln Met Gly Ala Val Asp Thr Ala Phe Asp Asp His Thr Ser Leu Phe

Asp Thr Gly Gly Ser Lys Gly Leu Thr Gly Asp Leu Val Glu Lys Ile 165 170 175

Pro Lys Met Thr Ile Thr Gly Asn Asn Asn Thr Asp Ala Ser Glu Asn

Thr Asp Ser Cys Ser Val Cys Leu Gln Asp Phe Gln Leu Gly Glu Thr

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Ser Glu Met Gly Glu Leu Pro Pro Ser Asn Cys Ser Met Asp Ser Phe Page 44

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MBI15 Sequence Listing.ST25 25 30

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Thr Asp Asp Thr Ala Glu Ser Cys Gly Lys Lys Gly Glu Lys Arg Pro

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Lys Ala Ala Ser Leu Glu Asp Glu Val Ala Arg Leu Arg Ala Val Asn 115 120 125

Gln Gln Leu Val Lys Arg Leu Gln Asn Gln Ala Thr Leu Glu Ala Glu

Val Ser Arg Leu Lys Cys Leu Leu Val Asp Leu Arg Gly Arg Ile Asp 145 150 160

Gly Glu Ile Gly Ser Phe Pro Tyr Gln Lys Pro Met Ala Ala Asn Ile

Pro Ser Phe Ser His Met Met Asn Pro Cys Asn Val Gln Cys Asp Asp 180 185 190

Glu Val Tyr Cys Pro Gln Asn Val Phe Gly Val Asn Ser Gln Glu Gly 195 200 205

Ala Ser Ile Asn Asp Gln Gly Leu Ser Gly Cys Asp Phe Asp Gln Leu 210 215 220

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aag atg gat caa aag act ctt cgt agg ctt gct caa aac cgt gag gca Lys Met Asp Gln Lys Thr Leu Arg Arg Leu Ala Gln Asn Arg Glu Ala 45 50 55	437
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atg gcc atg gca atg gga aag tta ggt aca ctc gaa gga ttt atc cgc Met Ala Met Ala Met Gly Lys Leu Gly Thr Leu Glu Gly Phe Ile Arg 270 275 280	1109
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gag tga aactgtattt tggtcacatg tcagctgtac aaaatccata tggacacaaa Glu 330	1309
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Met Ala Asp Thr Ser Pro Arg Thr Asp Val Ser Thr Asp Asp Asp Thr 15 Asp His Pro Asp Leu Gly Ser Glu Gly Ala Leu Val Asn Thr Ala Ala 25 Ser Asp Ser Ser Asp Arg Ser Lys Gly Lys Met Asp Gln Lys Thr Leu 45 Arg Arg Leu Ala Gln Asn Arg Glu Ala Ala Arg Lys Ser Arg Leu Arg 50 Lys Lys Ala Tyr Val Gln Gln Leu Glu Asn Ser Arg Leu Lys Leu Thr 65 Gln Leu Glu Gln Glu Leu Gln Arg Ala Arg Gln Gln Gly Val Phe Ile 95 Ser Gly Thr Gly Asp Gln Ala His Ser Thr Gly Gly Asn Gly Ala Leu	

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Met	Thr 210	Glu	Arg	Gln	Leu	Met 215	Gly	Ile	Asn	Asn	Leu 220	Gln	Gln	Thr	Ser	
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MBI15 Sequence Listing.ST25	
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•	. • • • •	., .														101/0
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						caa Gln										822
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						ttt Phe										918
						gaa Glu 220										966
						tca Ser					1.5				_	1014
						gct Ala										1062
						att Ile										1110
						aaa Lys										1158
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						ggt Gly										1254

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Lys Trp Glu Phe Leu Asn Asp Asp Phe Val Arg Gly Arg Pro Tyr Leu 85 90 95

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Glu Leu Gln Asn Gln Glu Gln Glu Arg Lys Glu Phe Glu Leu Gln Val 145 150 160

Thr Thr Leu Lys Asp Arg Leu Gln His Met Glu Gln His Gln Lys Ser 165 170 175

Ile Val Ala Tyr Val Ser Gln Val Leu Gly Lys Pro Gly Leu Ser Leu 180 185 190

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Leu Pro Pro Ser Ser Ser His Ile Glu Gln Val Glu Lys Leu Glu Ser 210 215 220

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Gln Gln G 305	lu Val		Ser (Glu	Arg	Arg	Asp	Val 315	Gly	Asn	Asp	Asn	Asn 320	•
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	MBI15 Sequence Listing.ST25															
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					ccg Pro											633
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Ser	Ser	Leu 85	Gly	Asn	Lys	Trp		15 S Arg						Pro	Gly	
						aag Lys 105										394
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						gtg Val										538
						aaa Lys										586
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						tac Tyr										682
						caa Gln										730
						tca Ser										778
						gaa Glu									gat Asp	826
						caa Gln 265				taa	gaaq	gagt	gaa 1	tatga	atcgta	879
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40

aac aac gag atg gta ttt atc cca cca aca agc gac gta gcc gtc aac

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96

144

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ccg Pro	cgt Arg	ctt Leu	agg Arg	tac Tyr 325	tta Leu	gat Asp	caa Gln	cgg Arg	ttg Leu 330	aga Arg	caa Gln	cag Gln	aga Arg	gct Ala 335	ttg Leu	1008
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Asn Leu Asn Pro Ser Thr Met Ser Asp Glu Asn Gly Lys Ser Leu Ser 115 120 125

Val His Gln His His Ser Asp Gln Ile Leu Pro Ser Ser Val Tyr Asn 130 135 140

Asn Asn Gly Asn Asn Gly Val Gly Phe Tyr Asn Asn Tyr Arg Tyr Glu 145 150 155 160

Thr Ser Gly Phe Val Ser Ser Val Leu Arg Ser Arg Tyr Leu Lys Pro 165 170 175

Thr Gln Gln Leu Leu Asp Glu Val Val Ser Val Arg Lys Asp Leu Lys 180 \$180\$

Leu Gly Asn Lys Lys Met Lys Asn Asp Lys Gly Gln Asp Phe His Asn 195 200 205

Gly Ser Ser Asp Asn Ile Thr Glu Asp Asp Lys Ser Gln Ser Gln Glu 210 215 220

Leu Ser Pro Ser Glu Arg Gln Glu Leu Gln Ser Lys Lys Ser Lys Leu 225 230 240

Leu Thr Met Val Asp Glu Val Asp Lys Arg Tyr Asn Gln Tyr His His 245 250 255

Gln Met Glu Ala Leu Ala Ser Ser Phe Glu Met Val Thr Gly Leu Gly 260 265 270

Ala Ala Lys Pro Tyr Thr Ser Val Ala Leu Asn Arg Ile Ser Arg His 275 280 285

Phe Arg Cys Leu Arg Asp Ala Ile Lys Glu Gln Ile Gln Val Ile Arg 290 295 300

Gly Lys Leu Gly Glu Arg Glu Thr Ser Asp Glu Gln Gly Glu Arg Ile 305 310 315 320

Pro Arg Leu Arg Tyr Leu Asp Gln Arg Leu Arg Gln Gln Arg Ala Leu 325 330 335

His Gln Gln Leu Gly Met Val Arg Pro Ala Trp Arg Pro Gln Arg Gly 340 345 350

Leu Pro Glu Asn Ser Val Ser Ile Leu Arg Ala Trp Leu Phe Glu His 355 360 365

Phe Leu His Pro Tyr Pro Lys Glu Ser Glu Lys Ile Met Leu Ser Lys 370 375 380

Gln Thr Gly Leu Ser Lys Asn Gln Val Ala Asn Trp Phe Ile Asn Ala 385 390 400

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Phe Gly Glu Ser Ala Glu Leu Leu Ser Asn Ser Asn Gln Asp Thr Lys 425

Lys Met Gln Glu Thr Ser Gln Leu Lys His Glu Asp Ser Ser Ser

Gln Gln Gln Asn Gln Gly Asn Asn Asn Asn Ile Pro Tyr Thr Ser 455

Asp Ala Glu Gln Asn Leu Val Phe Ala Asp Pro Lys Pro Asp Arg Ala

Thr Thr Gly Asp Tyr Asp Ser Leu Met Asn Tyr His Gly Phe Gly Ile

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Met Gly Leu Ala Thr Thr Thr Ser Ser Met Ser Gln Asp 111

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Tyr His His His Gln Gly Ile Phe Ser Phe Ser Asn Gly Phe His Arg
15 20 25 159

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MBI15 Sequence Listing.ST25

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acg Thr	ccg Pro	cga Arg	ttg Leu	cgt Arg	ttg Leu	cta Leu	gag Glu	cag Gln	Ser	ttg Leu	Arg	cag Gln	caa Gln	cga Arg	gcg Ala	1311

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35

Gln Ile Pro Val Tyr Glu Thr Ala Gly Met Leu Ser Glu Met Phe Ala 50 $\,$ 60

Tyr Pro Gly Gly Gly Gly Gly Ser Gly Gly Glu Ile Leu Asp Gln 65 70 75 80

Ser Thr Lys Gln Leu Leu Glu Gln Gln Asn Arg His Asn Asn Asn Asn 85 90 95

Asn Ser Thr Leu His Met Leu Leu Pro Asn His His Gln Gly Phe Ala

Phe Thr Asp Glu Asn Thr Met Gln Pro Gln Gln Gln His Phe Thr 115 120 125

Trp Pro Ser Ser Ser Ser Asp His His Gln Asn Arg Asp Met Ile Gly 130 135 140

Thr Val His Val Glu Gly Gly Lys Gly Leu Ser Leu Ser Leu Ser Ser 145 150 160

Ser Leu Ala Ala Ala Lys Ala Glu Glu Tyr Arg Ser Ile Tyr Cys Ala 165 170 175

Ala Val Asp Gly Thr Ser Ser Ser Ser Asn Ala Ser Ala His His His 180 185 190

Gln Phe Asn Gln Phe Lys Asn Leu Leu Leu Glu Asn Ser Ser Gln 195 200 205

His His His Gln Val Val Gly His Phe Gly Ser Ser Ser Ser Ser 210 215 220

Pro Met Ala Ala Ser Ser Ser Ile Gly Gly Ile Tyr Thr Leu Arg Asn 225 230 240

Ser Lys Tyr Thr Lys Pro Ala Gln Glu Leu Leu Glu Glu Phe Cys Ser 245 250 255

Val Gly Arg Gly His Phe Lys Lys Asn Lys Leu Ser Arg Asn Asn Ser 260 265 270

Asn Pro Asn Thr Thr Gly Gly Gly Gly Gly Gly Gly Ser Ser Ser Ser 275

Ala Gly Thr Ala Asn Asp Ser Pro Pro Leu Ser Pro Ala Asp Arg Ile 290 295 300

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Asp Arg Arg Tyr Asn His Tyr Cys Glu Gln Met Gln Met Val Val Asn 325 330 335

MBI15 Sequence Listing.ST25
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Leu Ala Gln Lys Ala Met Ser Arg His Phe Arg Cys Leu Lys Asp Ala 355 360 365

Val Ala Val Gln Leu Lys Arg Ser Cys Glu Leu Leu Gly Asp Lys Glu 370 375 380

Ala Ala Gly Ala Ala Ser Ser Gly Leu Thr Lys Gly Glu Thr Pro Arg 385 390 395 400

Leu Arg Leu Leu Glu Gln Ser Leu Arg Gln Gln Arg Ala Phe His His 405 410 415

Met Gly Met Met Glu Gln Glu Ala Trp Arg Pro Gln Arg Gly Leu Pro 420 430

Glu Arg Ser Val Asn Ile Leu Arg Ala Trp Leu Phe Glu His Phe Leu 435 440 445

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Arg Leu Trp Lys Pro Met Val Glu Glu Met Tyr Gln Gln Glu Ala Lys 485 490 495

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Asn Asn Phe Thr Val Ile Thr Ala Gln Thr Pro Thr Thr Met Thr Ser 530 535 540

Thr His His Glu Asn Asp Ser Ser Phe Leu Ser Ser Val Ala Ala Ala 545 550 560

Ser His Gly Gly Ser Asp Ala Phe Thr Val Ala Thr Cys Gln Gln Asp 565 570 575

Val Ser Asp Phe His Val Asp Gly Asp Gly Val Asn Val Ile Arg Phe 580 585 590

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305

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gac cga aaa tac Asp Arg Lys Tyr 90	ccg act ggt Pro Thr Gly	tta aga acg aat Leu Arg Thr Asn 95	cgt gct act aaa Arg Ala Thr Lys 100	gcc 401 Ala
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			tac aaa gga aga Tyr Lys Gly Arg 130	
			gag tat cga tta Glu Tyr Arg Leu	
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			ggt acg aag gag Gly Thr Lys Glu 180	
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gtg gcc gat ttt Val Ala Asp Phe	aaa act acc Lys Thr Thr 235	atg ttt ggt tcc Met Phe Gly Ser 240	gga tcg act aac Gly Ser Thr Asn 245	ttt 833 Phe
			cct ctg ttt cta Pro Leu Phe Leu 260	
			aat gaa gaa acc Asn Glu Glu Thr 275	
			gag agt gaa cta Glu Ser Glu Leu 290	
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Glu Pro Trp Asp Leu Pro Trp Lys Ala Lys Leu Gly Glu Lys Glu Trp 65 70 80

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Asn Arg Ala Thr Lys Ala Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys

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Phe Tyr Lys Gly Arg Ala Pro Lys Gly Val Lys Thr Asn Trp Val Met 130 140

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Asp Gly Thr Lys Glu His Met Ser Val Gly Leu Pro Pro Leu Met Asp

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Thr Leu Gly Gly Leu Leu Ser His Val Thr Tyr Phe Ser Asp Gln Thr 210 215 220

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MBI15 Sequence Listing.ST25

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102

103

104

105

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40

45

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Ala Phe Ser Ser Tyr Gln Ala Leu Gly Gly His Lys A	la Ser His Arg 110
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Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Glu G 165 170	ly Lys Asn Gly 175
Gly Gly Val Ser Ser Ser Val Ser Asn Ser Glu Asp V 180 185	al Gly Ser Thr 190
Ser His Val Ser Ser Gly His Arg Gly Phe Asp Leu A 195 200 2 Page 71	asn Ile Pro Pro 205

MBI15 Sequence Listing.ST25

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678

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MBI15 Sequence Listing.ST25

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Val Asn Tyr Lys Gly Lys Ser Tyr Ser Leu Lys Gly Cys Ile Ser Asp 100 105 110

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Leu Cys Pro Leu Asp His Glu Thr Glu Gly Lys Ile Ile Asp Asp Glu 195 200

Ile Asn Arg Ile Leu Val Arg Pro Leu Val His Gly Ala Lys Leu His 210 215 220

Ala Val Ile Asp Ala Cys Asn Ser Gly Thr Val Leu Asp Leu Pro Phe 225 230 235 240

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Gly Lys Asn Thr Gly Ala Met Thr Tyr Ser Phe Ile Lys Ala Val Lys 290 295 300

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Ser Ala Ile Arg Glu Ala Gln Ser Arg Leu Ala Phe Asn Gly Asp Tyr Page 76

MBI15 Sequence Listing.ST25 330 335

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INTERNATIONAL SEARCH REPORT

Interna al application No.
PCT/US00/31418

			PC1/US00/31418)
A. CLAS	SSIFICATION OF SUBJECT MATTER			
IPC(7)	: A01H 1/00, 5/00; C12N 5/14, 15/82			
US CL	: 435/320.1, 419, 468; 800/278, 279, 287, 301,			
	International Patent Classification (IPC) or to both n	ational cla	assification and IPC	 · · · · · · · · · · · · · ·
B. FIEL	DS SEARCHED			
Minimum do	cumentation searched (classification system followed	by classif	ication symbols)	
	35/320.1, 419, 468; 800/278, 279, 287, 301, 305-31			
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<u> </u>				
Documentation	on searched other than minimum documentation to the	e extent th	at such documents are include	d in the fields searched
				····
Electronia da	ata base consulted during the international search (nam	ne of data	hase and where practicable of	oarch tarms used)
	AT; STN, Agricola, CaPlus, Biosis, Embase	ne or uata	base and, where practicable, s	carcii terms useu)
Chot, Oder	11, 0111, figiteois, Carias, Biosis, Bilioase		•	
	UMENTS CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where an			Relevant to claim No.
X	WO 97/47183 A1 (PURDUE RESEARCH FOUND	ATION) 1	8 December 1997	1-9, 12, 13, 25
;	(18.12.1997), entire reference.		j	10 11 04 05
Y				10, 11, 26, 27
x	US 5,939,601 (KLESSIG et al) 17 August 1999 (17	.08.1999),	, entire reference.	1-9, 12, 13, 25
Υ				10, 11, 26, 27
A	Database Genbank on NCBI, US National Library	of Medicir	ne. (Bethesda, MD. USA)	1-13, 25-27
'	No. AB009055, SATO, S. et al 'Strucural analysis			,
	IV. Sequence features of the regions of 1,456,315 b			
	assigned P1 and TAC clones. 27 December 2000,			
	41-54, see bases 16,003-16,490, 16,571-16,683 and			
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1				
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, 	r documents are listed in the continuation of Box C.		See patent family annex.	
• s	pecial categories of cited documents:	"T"	later document published after the into	
"A" document	defining the general state of the art which is not considered to be		date and not in conflict with the applic principle or theory underlying the inve	
	ular relevance	647		
"E" earlier au	oplication or patent published on or after the international filing date	"X"	document of particular relevance; the considered novel or cannot be consider	
			when the document is taken alone	
	t which may throw doubts on priority claim(s) or which is cited to the publication date of another citation or other special reason (as	-Y"	document of particular relevance; the	claimed invention cannot be
specified			considered to involve an inventive ste	p when the document is
"O" documen	referring to an oral disclosure, use, exhibition or other means		combined with one or more other such being obvious to a person skilled in the	
	t published prior to the international filing date but later than the date claimed	*&*	document member of the same patent	tamuly
Date of the	actual completion of the international search	Date of	mailing of the international sea	rch report
			19 MAR 21	nol.
	2001 (23.02.2001)	Authoria	ted officer	77
	nailing address of the ISA/US muissioner of Patents and Trademarks	, Authoriz		RY J. DEY
	mussioner of Patents and Trademarks	David K		N. SPECIALIST
Wa	shington, D.C. 20231	Talaska		
Facsimile N	o. (703)305-3230	i elepno:	ne No. 703-308- TEMMOLO	IT VENIEN 1000

INTERNATIONAL SEARCH REPORT

Inter nal application No.

PCT/US00/31418

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)		
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:		
1.		Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2.		Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.	6.4(a).	Claim Nos.: 14 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)		
		ional Searching Authority found multiple inventions in this international application, as follows: ontinuation Sheet
1.		As all required additional search fees were timely paid by the applicant, this international search report covers all
		searchable claims.
2.	Ш	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.		As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.	\boxtimes	No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13 & 25-27 and SEQ ID NOs 1&2
Rem	nark on	Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Ittu.....nal application No.

PCT/US00/31418

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXIX, claim(s) 1-14 and 25-27, drawn to a transgenic plant having modified seed characteristics, polynucleotides and vectors for producing said transgenic plant and a method of making said transgenic plant. Applicant must elect one pair of sequences (one nucleic acid and the corresponding amino acid translation) to be examined, i.e. SEQ ID NO: 1 and 2 in Group I, SEQ ID NO: 3 and 4 in Group II, SEQ ID NO: 5 and 6 in Group III, etc.

Group XXX, claim(s) 15-17, drawn to a method of identifying a factor that is modulated.

Group XXXI, claims(s) 18, drawn to a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide.

Group XXXII, claims(s) 19 and 20, drawn to an integrated computer system.

Group XXXIII, claim(s) 21-24, drawn to a method for identifying a polynucleotide sequence comprising selecting a nucleic acid sequence from a database that meets a selected sequence criteria.

The inventions listed as Groups I-XXXIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I-XXXIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-XXIX are drawn to a transgenic plant and a method of producing said plant with a nucleic acid sequence. The methods of Groups I-XXIX differ from each other in that they are directed to a plant transformation method and transgenic plant with a structurally and functionally distinct nucleic acid sequence which encodes a structurally and functionally distinct amino acid sequence. In addition, Groups XXX, XXXI and XXXIII are different methods from any of Groups I-XXIX in that they have different method steps and different end products, and Group XXXII requires a computer system. Thus, there is no single special technical feature, which links the inventions of Groups I-XXXIII under PCT Rule 13.2.